

A7

43. (Amended) A method of culture of ES cells, comprising expressing in the ES cells a compound which selectively promotes self-renewal of the ES cells, inhibits propagation or survival of cells other than ES cells, or both promotes self renewal of the ES cells and inhibits propagation or survival of cells other than ES cells.

Please add the following new claims 46-50.

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46. (New) A method according to Claim 12 wherein the second compound is PD098059.

47. (New) A composition according to Claim 18 wherein the second compound is PD098059.

48. (New) A culture medium according to Claim 28 wherein the second compound is PD098059.

49. (New) A method according to Claim 34 wherein the compound that selectively inhibits propagation or survival of cells other than ES cells is PD098059.

50. (New) A method according to Claim 12 wherein the first compound is LIF and is at a concentration of 5 Units per milliliter (U/ml).

REMARKS

Applicants have amended the claims to render the claim language more clear. Claims 46-50 are added to claim specific embodiments of the invention. Support for the amendment to claims 12 and 28 can be found in the specification at, for example, page 7, paragraph 5. Claims 46-49 are supported throughout the specification, for example at page 5, paragraph 4. Support for claim 50 can be found, for example in Figure 4 and at page 15 of the specification. None of the amendments or new claims constitutes new matter.

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If there is any fee due in connection with the filing of this Preliminary Amendment, please charge the fee to Deposit Account No. 06-0916.

Respectfully submitted,

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APPENDIX

Amended Claims 12, 14-20, 21, 27-28, 30-33, 36, 39 and 43; and add new claims 46-50.

12. A method of culture of ES cells comprising maintaining a culture of ES cells in the presence of a synergistic combination of:-

- (a) a first compound that promotes propagation or survival of ES cells; and
- (b) a second compound that inhibits propagation or survival of cells other than ES cells.

14. A method according to Claim 12 [or 13] wherein the second compound [(b)] selectively inhibits a signalling pathway essential to propagation or survival of cells other than ES cells.

15. A method according to [any of] Claim[s] 12 [to 14], wherein the second compound [(b)] is selected from the group consisting of: compounds which inhibit activity of the enzyme SHP-2[,]; compounds that inhibit the ras/MAPK cascade[,]; compounds that inhibit MEK[,]; compounds that inhibit a mitogen activated protein kinase; [or] and compounds that inhibit cyclin dependent entry into S-phase of non-ES cells.

16. A method according to [any of] Claim[s] 12 [to 15] wherein the first compound [(a)] is a cytokine that activates the cytokine receptor gp130 in ES cells.

17. A method according to Claim 12 [16], wherein the first compound is [the cytokine is] LIF.

18. A composition for selective culture of ES cells, comprising a synergistic combination of (a) a first compound that inhibits differentiation of ES cells, and (b) [a synergistic amount of] a second compound that inhibits propagation or survival of cells other than ES cells.

19. A composition according to Claim 18 wherein the second compound [(b)] selectively inhibits a signalling pathway essential to propagation or survival of cells other than ES cells.

20. A composition according to Claim 18 [or 19] wherein the second compound [(b)] is selected from the group consisting of: compounds which inhibit activity of the enzyme SHP-2[,]; compounds that inhibit the ras/MAPK cascade[,]; compounds that inhibit MEK[,]; compounds that inhibit a mitogen activated protein kinase; [or] and compounds that inhibit cyclin dependent entry into S-phase of non-ES cells.

21. A composition according to [any of] Claim[s] 18 [to 20] wherein the first compound [compounds (a)] is a cytokine that binds to the cytokine receptor gp130[, such as LIF].

27. A culture medium according to Claim 25 [or 26] wherein the second compound is selected from the group consisting of: compounds which inhibit activity of the enzyme SHP-2[,]; compounds that inhibit the ras/MAPK cascade[,]; compounds that inhibit MEK[,]; compounds that inhibit a mitogen activated protein kinase; [or] and compounds that inhibit cyclin dependent entry into S-phase of non-ES cells.

28. A culture medium for culture of ES cells and comprising (a) a first compound that promotes proliferation of ES cells, and (b) a second compound that inhibits propagation or survival of cells other than ES cells, wherein the combination of the first and second compounds is synergistic.

30. A culture medium according to Claim 28 [or 29], wherein the second compound [(b)] selectively inhibits a signalling pathway essential to propagation of cells other than ES cells.

31. A culture medium according to [any of] Claim[s] 28 [to 30], wherein the second compound [(b)] is selected from the group consisting of: compounds which inhibit activity of the enzyme SHP-2[,]; compounds that inhibit the ras/MAPK cascade[,]; compounds that inhibit MEK[,]; compounds that inhibit a mitogen

activated protein kinase; [or] and compounds that inhibit cyclin dependent entry into S-phase of non-ES cells.

32. A culture medium according to [any of] Claim[s] 28 [to 31], wherein the first compound [(a)] is a cytokine that activates the cytokine receptor gp130 in ES cells.

33. A culture medium according to Claim [32] 28, wherein the first compound is [the cytokine is] LIF.

36. A method according to Claim 34 [or 35] comprising developing an embryo *in vivo*, harvesting the embryo prior to pro-amniotic cavity formation and isolating cells therefrom.

39. A method according to [any of] Claim[s] 34 [to 38], wherein the compound that selectively inhibits propagation or survival of cells other than ES cells is selected from the group consisting of: compounds which inhibit activity of the enzyme SHP-2[,]; compounds that inhibit the ras/MAPK cascade[,]; compounds that inhibit MEK[,]; compounds that inhibit a mitogen activated protein kinase; [or] and compounds that inhibit cyclin dependent entry into S-phase of non-ES cells.

43. A method of culture of ES cells, comprising expressing in the ES cells a compound which selectively promotes self-renewal of the ES cells, [and/or] inhibits propagation or survival of cells other than ES cells, or both promotes self renewal of the ES cells and inhibits propagation or survival of cells other than ES cells.

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